Indomethacin inhibits the in vivo formation of the lipoxygenase product HETE (12-hydroxy-5,8,10,14-eicosatetraenoic acid) during granulomatous inflammation in the rat

P. C. BRAGT*, I. L. BONTA, *Department of Pharmacology, Faculty of Medicine, Erasmus University Rotterdam, P.O. Box 1738, Rotterdam, The Netherlands*

During both acute and chronic phases of carrageenan-induced granulomatous inflammation in the rat, arachidonic acid is mainly converted in vivo into polyunsaturated hydroxy fatty acids and PGE_2 (Bragt & Bonta 1979). The hydroxy fatty acids HETE and HHT (12-hydroxy-5,8,10-heptadecatrienoic acid) are chemotactic to polymorphonuclear (PMN) leucocytes (Turner et al 1975; Goetzl & Gorman 1978). The formation of HHT can be blocked by non-steroidal anti-inflammatory drugs (NSAIDs) and thromboxane synthetase inhibitors (Diczfalusy et al 1977), whereas the lipoxygenase-dependent formation of HETE is not blocked by the NSAIDs, but rather stimulated, at least in human platelets (Hamberg et al 1974). In addition, aspirin, phenylbutazone and indomethacin, when given orally 1 h before sponge implantation in the rat, significantly inhibit the migration of PMN leucocytes into the 5 h sponge exudate (Ford-Hutchinson et al 1975). More recently, it was demonstrated that low doses of indomethacin and aspirin stimulate the migration of PMN leucocytes into the inflamed area 24 h after the implantation of sponges, but high doses inhibit. Moreover, the migration was also inhibited by the compound BW755C (3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline), an inhibitor of both lipoxygenase and cyclo-oxygenase (Higgs et al 1979). Higgs (1979) subsequently suggested that high doses of aspirin and indomethacin might inhibit lipoxygenase activity.

We have investigated the effects of indomethacin on the in vivo metabolism of arachidonate, 24 h and 8 days after the implantation of cannulated Teflon chambers and the induction of granuloma growth around the chamber by the injection of carrageenan.

Cannulated Teflon chambers were implanted, subdermally, into the backs of male Wistar rats (190–220 g), divided into two groups of 5 animals, as previously described (Bragt et al 1979). Inflammation was induced immediately afterwards by the injection through each cannula of 1 ml 1% (w/v) carrageenan in 0·9% NaCl (saline). One group of rats received a daily dose of indomethacin (200 μg) directly into the chamber, whereas the other group received the vehicle. Four hours after these injections, on days 1 and 8 of granuloma development, sodium [1-^{14}C]arachidonate was injected into the chambers. The in vivo incubation procedure, the recovery of the inflammatory exudate by perfusion and the identification and quantification of the arachidonate metabolites formed, were carried out as previously described (Bragt et al 1979). One sample of the labelled arachidonate was aerobically incubated for 1 h at 37 °C and stirred at 500 rev min^{-1}, as a control for the autoxidative formation of prostaglandin-like products.

Effects of indomethacin on arachidonate metabolism. The results of the present set of experiments are summarized in Table 1. The local injection of indomethacin, immediately after the induction of inflammation, resulted, after 24 h, in a significant decrease in the formation of PGE_2, $PGF_{2\alpha}$, TXB_2 and HHT. While the synthesis of 6-keto$PGF_{1\alpha}$, the metabolite of prostacyclin, was unaltered by indomethacin, the synthesis of the lipoxygenase product HETE was markedly inhibited, to a level approaching the amount of HETE-like material formed during autoxidation.

When the local administration was continued daily up until day 8 of granuloma development, only the formation of PGE_2 was significantly inhibited by indomethacin. The formation of all other metabolites, including HETE, was not significantly reduced at this stage. Results not included in Table 1 show that, whereas on day 1 there was an increased conversion of the total injected arachidonate after indomethacin treatment (24·8 ± 2·2% in control and 16·0 ± 1·0% in drug treated rats, $P < 0.01$, one-tailed Student's *t*-test), arachidonate conversion was unchanged by the drug on day 8. Furthermore, the amount of arachidonate incorporated into the phospholipid ($R_F = 0$) fraction on day 1 was significantly enhanced by treatment of the rats with indomethacin (14·1 ± 1·4% in control animals against a mean ± s.e.m. of 10·2 ± 0·7% of total radioactivity in indomethacin-treated rats ($P < 0.025$). No difference in this parameter was observable on day 8 of inflammation. Similar changes were observed in the glyceride fraction (data not shown).

Except for the hydroxy fatty acids, HHT and HETE, the capacity for biosynthesis of the arachidonate metabolites was more pronounced on day 8 than on day 1 (Table 1). On day 8, the animals were killed and the granulomata were dissected out and weighed. There was almost no reduction in the mean wet granuloma weight following indomethacin treatment (2·3 ± 0·3 g against 2·4 ± 0·1 g in control rats, $P > 0.4$), although the formation of PGE_2 was still significantly inhibited at this stage of inflammation (Table 1). Thus, granuloma growth and the formation of PGE_2 can be influenced independently of each other, as recently reported by Bonta et al (1979).

Our data clearly show that the daily administration of indomethacin (200 μg, locally), inhibits the biosynthesis

* Correspondence.

Table 1. Effects of daily administration of indomethacin (200 μg, locally) on the in vivo metabolism of sodium [1-^{14}C]arachidonate on days 1 and 8 of carrageenan-induced granulomatous inflammation in the rat.

	Product formed as a percentage of total plate radioactivity				
	Day 1		Day 8		
Metabolite	Vehicle	Drug	Vehicle	Drug	Autoxidation
PGE_2	0·88 ± 0·04	0·50 ± 0·09**	5·30 ± 2·40	0·72 ± 0·10*	0·14
$PGF_{2\alpha}$	0·28 ± 0·04	0·20 ± 0·02*	0·73 ± 0·31	0·24 ± 0·02	0
6-ketoPGF$_{1\alpha}$	0·34 ± 0·07	0·29 ± 0·09	0·83 ± 0·63	0·10 ± 0·03	0
TXB_2	0·34 ± 0·03	0·25 ± 0·03*	0·85 ± 0·31	0·42 ± 0·14	0
HHT	1·86 ± 0·25	1·16 ± 0·10*	0·68 ± 0·11	0·72 ± 0·12	0·35
HETE	1·27 ± 0·04	0·71 ± 0·06**	0·63 ± 0·17	0·50 ± 0·10	0·71

Values represent means ± s.e.m. of 5 rats. Significance of the differences between indomethacin and vehicle treated rats was tested by the one-tailed Student's t-test (*$P < 0·05$ and ** $P < 0·01$). The values ranked in the last column represent the formation of products during the autoxidation of arachidonate with identical R_F-values as the metabolites formed in vivo.

of most arachidonate metabolites during the acute phase of inflammation, whereas in the chronic phase the inhibition is less pronounced. This finding is consistent with the results of other investigators that indomethacin is a more effective inhibitor of granuloma growth during the acute, rather than the chronic phase (Fukuhara & Tsurufuji 1969). Furthermore, our experiments confirm the observation that the pool of cyclo-oxygenase involved in the ultimate synthesis of prostacyclin is relatively insensitive towards inhibition by low doses of NSAID in vivo (Basista et al 1978).

Although the inhibition of HETE formation on day 1 might be the result of a direct inhibition of lipoxygenase(s) by indomethacin, two other explanations are also possible, as indomethacin has not been reported to be a lipoxygenase inhibitor in vitro (Hamberg et al 1974). Indomethacin inhibits the migration of leucocytes (Ford-Hutchinson et al 1975) and this inhibition may lead to a reduced amount of arachidonate converting enzymes at the inflamed site and a reduced formation of metabolites. The insensitivity of the formation of arachidonate metabolites on day 8 towards inhibition by indomethacin, may be due to the relative dominance of other cell types in this phase of inflammation.

It is of particular interest that at least a part of the injected arachidonate is incorporatedin to phospholipids and indomethacin increases the amount of arachidonate incorporated into this fraction on day 1. Kaplan et al (1978) have shown that indomethacin, in very low concentrations, inhibits the phospholipase A_2 of rabbit PMN leucocytes. If the major part of the arachidonate administered in our inflammation model is indeed incorporated into membranes and subsequently released by a phospholipase, it is not inconceivable that the inhibition by indomethacin of the formation of HETE and other products during the acute stage of inflammation is due to the inhibition of phospholipase A_2, resulting in a decreased availability of arachidonic acid for cyclo-oxygenase and lipoxygenase.

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Note added in proof:

It has recently been shown that aspirin-like drugs inhibit the formation of HETE by human platelet microsomes, owing to the inhibition a HPETE peroxidase (Siegel, M. I., McConnell, R. T., Cuatrecasas, P. (1979) Proc. Natl. Acad. Sci. U.S.A. 76: 3774–3778).

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